214659US-0

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :

NORIKO MIWA ET AL : ATTN: APPLICATION DIVISION

SERIAL NO: NEW APPLICATION :

FILED: HEREWITH :

FOR: METHOD FOR MODIFYING RAW MATERIAL MILK AND DAIRY PRODUCT PREPARED BY USING THE MODIFIED RAW MATERIAL MILK

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to a first examination on the merits, please amend the above-identified application as follows:

IN THE SPECIFICATION

Page 10, lines 21-23, please replace the paragraph with the following paragraph:

Fig. 1 shows a sodium dodecyl sulfate(SDS)-polyacrylamide gel electrophoresis pattern illustrating an improvement in the reactivity of raw material milk to TG by the addition of reduced glutathione (Example 1).

Page 11, lines 1-3, please replace the paragraph with the following paragraph:

Fig. 3 shows an SDS-polyacrylamide gel electrophoresis pattern illustrating an improvement in the reactivity of raw material milk to TG by the addition of sodium ascorbate (Example 2).

Page 15, lines 3-18, please replace the paragraph with the following paragraph:

The TG to be used according to the present invention is an enzyme which catalyzes the acyl group transfer reaction which acyl group is present in the γ-carboxyamide group of a glutamine residue in a protein or peptide chain. When this TG acts upon the ε-amino group of a lysine residue as an acyl acceptor in a protein, ε-(γ-glutamyl)-lysine bonds are formed in and between the molecules of the protein or intramolecularly and intermolecularly. By these crosslinks, strong networks are formed among the molecules of a milk protein in raw material milk, whereby modified raw material milk having such properties as high gel formability, high viscosity and high water-holding capacty is produced, and, in turn, a dairy product having improved physical properties can be produced by using the modified raw material milk. TG which is the enzyme to be used according to the present invention can be any TG as long as it has transglutaminase activity, and known TGs can be used.

Page 15, line 19 to page 16, line 6, please replace the paragraph with the following paragraph:

TGs can be classified into calcium-independent TG and calcium-dependent one, and both types of TGs can be used according to the present invention. Illustrative examples of the former include those derived from microorganisms such as TG derived from Actinomycetes (refer to Japanese Patent No. 2,572,716), TG derived from bacillus subtilis (refer to Japanese Patent Application Laid-Open No. 137254/1999), and the like. Illustrative examples of the latter include TG derived from a guinea pig's liver (refer to Japanese Patent No. 1,689,614), TG derived from microorganisms such as Oomycetes and the like (refer to WO96/22366), TG

derived from animal blood such as bovine blood, swine blood, and the like, TG derived from fishes such as salmon and sea bream (N. Seki et al., Nippon Suisan Gakkaishi (1990) 56, 125 to 132), and TG derived from oysters (U.S. Patent No. 5,736,356), and the like.

Page 17, lines 3-14, please replace the paragraph with the following paragraph:

As has already been described above, it is known that TGs have a variety of origins. Depending on the origins, some TGs have the substrate specificity which inhibits defining the activity by the above hydroxamate method. In that case, the unit may be defined by a different method. Regardless of which activity-measuring method is used to define the unit, the amount of the TG is included within the range of the amount of TG which can be added according to the present invention as long as the first-mentioned amount is substantially the amount which exhibits what is called "the effect of improving the physical properties of a dairy product" according to the present invention.

Page 17, line 25 to page 18, line 16, please replace the paragraph with the following paragraph:

The degree of crosslinking of milk proteins with the use of TG, in other words, the degree of modification of milk with the use of TG, can be appropriately adjusted by such reaction conditions as the amount, reaction time, reaction temperature and the like, concerning TG, depending on the physical properties of the desired dairy product. The degree of crosslinking of milk proteins can be examined by a quantitative method and a qualitative method. Illustrative examples of the quantitative method include the analysis of the quantity of the ε-(γ-glutamyl) lysine bonds, i.e., G-L bonds, in proteins by liquid chromatography (Griffin and Wilson, Molecular and Cellular Biochemistry (1984), 58, 37 to 49) and the measurement of the amount of ammonia produced by the crosslinking reaction (Ikura et al., Agricultural and Biological Chemistry, (1980), 45, 2587 to 2592). Illustrative

examples of the qualitative method include a method of examining the degree of crosslinking and the molecular weight by electrophoresis (Traore and Meunier, Journal of Agricultural and Food Chemistry, (1991), 39, 1892 to 1890).

Page 19, lines 16-22, please replace the paragraph with the following paragraph:

The present invention is excellent in that the required amount of a reducing agent is at a realistic level where the reducing agent can be used in food. For example, when a yeast extract containing glutathione in high concentration or an ascorbate is used according to the present invention, they can be used in such amount that they hardly affect the taste of foods such as milk and dairy food.

Page 20, lines 2-17, please replace the paragraph with the following paragraph:

Reduced glutathione was added to 5 ml of low-temperature-sterilized cow milk (kept at 63°C for 30 minutes to be sterilized; non-fat milk solid: 8.4%; milk protein: 3.1%; milk fat: 3.6%) in such amount that the content of the reduced glutathione in the milk would be 0 to 0.2 mM, and together with the reducing agent, an enzyme preparation of TG ("ACTIVA" TG, specific activity: 1,000 units/gram of the preparation, product of AJINOMOTO CO., INC.) was added in an amount of 2 units per 1 gram of milk proteins. The reaction was carried out by keeping the resulting mixture at 40°C for 3 hours. The degree of crosslinking of the proteins was examined by SDS-polyacrylamide gel electrophoresis. The detection of the proteins was carried out by immersing a gel containing migrated proteins in a solution containing dye (Coomassie brilliant blue) which specifically bonds to the proteins caused to migrate into the gel by electrophoresis and then destaining the gel.

Page 25, lines 16-22, please replace the paragraph with the following paragraph:

To each of the thus-obtained modified raw material milks, a commercial lactic acid bacteria starter "Yo Flex YC-370" (product of Chrischan Hansen's Laboratories) was added in

the proportion of 0.0063% based on the raw material milk. After the resulting milks were charged into containers, they were respectively fermented at 44°C until the pH reached 4.5, whereby yogurts were prepared.

REMARKS

Respectfully submitted,

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